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Title: Critical Fluid Options for Isolating and Processing
Agricultural and Natural Products

Author(s): Jerry W. King

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Critical fluid technology options for isolating and processing agricultural and natural products

Jerry W. King

²*Los Alamos National Laboratory, Los Alamos, New Mexico 87545 USA*

Abstract

The use of pressurized fluids in their sub- or supercritical state provide a versatile medium that are compatible for isolating and processing naturally-derived products in an environmentally benign manner. In addition, by proper selection of the extracting fluid, one can obtain extracts/fractions for direct use as food or nutraceuticals, medical and drug applications, and extracts having cosmetic utility. In this presentation both discrete and serial combinations of sub- and supercritical carbon dioxide with or without ethanol, and sub-critical water with ethanol will be shown to effective solvent combinations for many processes. Supercritical carbon dioxide is well documented as an effective agent for the extraction and processing of many natural product matrices, however addition of up to 20 mole % of GRAS-approved ethanol increases the solubilization of polar solutes . Multi-phase systems consisting of pressurized carbon dioxide with ethanol at pressures beyond 70 MPa also extend the range of applicability of this "green" solvent combination. Water in its hot compressed state (i.e., sub-critical water) provides an alternative solvation medium for the extraction and reaction of polar compounds, while maintaining the naturalness of the resultant extracts. Liquids such as ethanol under pressure and at temperatures above its normal boiling, cover a range of dielectric constant or solubility parameter that are normally attained by using polar and problematic organic solvents. Results will be presented that demonstrate how solute solubility behavior can be altered by application of temperature, addition of ethanol, or naturally-compatible additives to the aqueous phase. Applications of sub-critical water-based extractions or reactions will be demonstrated for naturally-derived compounds, such as anthocyanins from berries at temperatures from 100-150 C. High recoveries (>90%) are achieved for these thermally-sensitive compounds by maintaining a high superficial velocity of the compressed water through the extraction vessel (approx. 0.1 cm/sec) and applied pressures in excess of those required to suppress the boiling tendency of water. Processing schemes based upon the above discrete processes will be illustrated for the selective extraction and reaction of compounds from natural product matrices. When coupled with columnar fractionation, chromatography, and membrane fractionation, the range of applicability of these novel compressed solvents is significantly extended. These combinations than allow the tailoring of the derived extract for its end-use application.

1. Introduction

The application of supercritical fluids (SF) and similar media for the processing of agricultural or natural products has traditionally focused on the extraction mode utilizing carbon dioxide in its supercritical (SC-CO$_2$) or liquid (LCO$_2$) state. Beginning in the mid-1980's, options other than varying the extraction fluid's density in the SFE mode were developed, such as columnar and

chromatographic techniques, which facilitated SF-derived extracts or products having more specific composition and properties. This development was followed by the advent of conducting reactions (SFR) in the presence of SFs as documented in the literature [1,2]. Further examination of alternative fluids, such as subcritical water have expanded the "natural" fluid base available to the processor of agriculturally- derived products. Therefore it should be possible to process natural product matrices utilizing a series of pressurized fluids such as suggested by the sequence below:

$$SC\text{-}CO_2 \text{ or } LCO_2$$
$$\downarrow$$
$$SC\text{-}CO2/Ethanol \text{ or } H_2O$$
$$\downarrow$$
$$\text{Pressurized } H_2O \text{ or } H_2O/Ethanol$$

The above sequence suggests that some degree of selective solvation should be possible, with SC-CO$_2$ or LCO$_2$ extracting non-polar solutes followed by the enhanced solubilization of more polar moieties via the addition of ethanol to the SF. Processing with pressurized water, i.e., subcritical H$_2$O (sub-H$_2$O) expands the range of extractable solutes into the polar solute range with selectivity being controlled by the temperature of extraction or addition of ethanol. Depending on the composition or morphology of the natural product being extracted, there is no reason in theory or practice that the above process could not be done in the reverse order.

Therefore by combining such discrete unit processes such as SFE, fractionation (SFF), or SFR in various combinations with a matrix of extraction fluids, a number of coupled processing options can be devised yielding unique products as described below in Figure 1:

Combing Different Processes
Use of Multiple Fluids
Different Sequencing of Processes
Different Conditions and Phase
$$\downarrow$$
Multiple Extracts/Products

Figure 1. Coupled Options Using Critical Fluids

A more detailed example of this coupled processing concept is cited below in Table I for the processing of citrus oils using pressurized fluids. Here six discrete unit processes are listed which include standard SFE with SC-CO$_2$, SFF employing stage-wise pressure reduction, SFF as practiced

using column-based deterpenation [3], supercritical fluid chromatography (SFC), another variant of SFF called subcritical water deterpenation [4], and utilization of a SC-CO$_2$ or LCO$_2$ with a permselective membrane described by Towsley et al. [5]. As shown in Table I, combinations of these processes can be coupled to advantage to allow a total processing scheme to be conducted using critical fluids.

For example, a combination of processes (1) and (2) in Table I could be combined to yield a more specific composition in the final extract. Unit process (1) if conducted by sequentially increasing the extraction density when coupled with a sequence of let down pressures (unit process 2) can amplify the SFF effect. Likewise, by combining unit process (1) using SC-CO$_2$ followed by application of unit process (2) utilizing subcritical H$_2$O to deterpenate the extract from unit process (1), will yield a more specific final product from the starting citrus oil. To obtain a more enriched and/or concentrated product from the latter process, one could add on unit process (6), a supercritical fluid membrane-based separation of the aqueous extract/fractions from unit process (5) as indicated below (Table I).

Table I. Coupled Processing Options for Citrus Oils Using Pressurized Fluids

Process: (1) SFE (SC-CO$_2$)
(2) SFF (SC-CO$_2$) – Pressure Reduction
(3) SFF (SC-CO$_2$) – Column Deterp[1]
(4) SFC (SC-CO$_2$/Cosolvent)
(5) SFF – (Subcritical H$_2$O Deterp[1]
(6) SFM – (Aqueous Extract/SC-CO$_2$)

Combinations: (1) + (2)
(1) + (2) + (3)
(1) + (4)
(1) + (5)
(1) + (5) + (6)

[1] Deterp. = Deterpenation

Several other combinations of the above unit processes will be noted in the Results and Discussion section, including an integrated unit process scheme for the enrichment of fish oil esters. The author and his colleagues have also developed several combinations of processes for the production of oleochemical industrial products [6,7,8,9] which are cited in the literature. Currently there is only one industrial company which offers both SC-CO$_2$ and sub-H$_2$O processing; Express Separations Inc. in England (www.expsep.co.uk).

2. Experimental

The experimental conditions for coupling the discrete unit processing steps to obtain high purity phospholipid extracts have been described in the literature [10,11] and will not be repeated here. A description of our recent application of sub-H_2O for the extraction of anthocyanins from berry substrates follows.

2.1 Experimental apparatus

The experimental apparatus used to conduct sub-H_2O extraction on berry substrates is shown in Figure 2. It consists of a modified Applied Separations Inc. (Allentown, PA) Spe-ed pumping unit feeding water from a reservoir into a extraction vessel (cell) contained in a thermo-regulated oven (Model 3710A, ATS, Inc., Butler, PA). The extraction cell was a 316 SS, 1" o.d., 9/16"i.d., approximately 55 mL in volume.

As shown in Figure 2, the water is pumped through an equilibration coil contained in the oven to bring it into its subcritical state at temperatures above its normal boiling point under pressure, and then passed through the extraction cell before exiting the oven into a cooling bath reservoir (Model 801, Polyscience, Inc., Nile, IL). Back pressure was maintained on the system with the aid of a micrometering valve which also allowed adjustment of the water flow rate. Aqueous extracts were collected after exiting the micrometering valve.
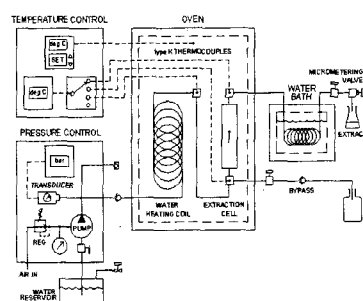

Figure 2. Subcritical water extraction system for extracting anthocyanins from berry substrates.

The first thermocouple in Figure 2 was connected to the temperature controller (Part No. CN4800, Omega Engineering, Stanford, CT) which regulated the oven temperature while the other thermocouples were connected to a digital meter to obtain an accurate reading of the water temperature, both before and after the extraction cell.

2.2 Extraction procedure

Samples of various fruit berries and their by-products (pomaces) were placed in the extraction cell and the oven heated to temperatures between 110-160°C. Both deionized and Milli-Q-purified neat water as well as acidified water (0.01% HCl, pH ~ 2.3) were fed at a rate of 24 mL/min at a constant pressure of 40 bar (580 psi). This pressure was well in excess of that required to prevent the formation of steam within the extraction cell. Incremental samples were obtained every 60-80g of aqueous solution expelled from the extractor over a 40 min time interval, however extracts were not taken until the cell was at the desired extraction temperature and pressure.

Color was monitored visually to an approximate equivalent of 20 ppm of cyanin-3-glucoside (a specific anthocyanin). Extract samples were analyzed by the HPLC procedure described by Skrede et al. [12]. The efficiency of the sub-H_2O extraction was compared to results obtained using a 70% ethanolic extract. The control sample was extracted with 70% ethanol in water for 40 min using sonication and washed with excess ethanol to remove .any remaining color from the berry substrate. Because of the extreme sensitivity of anthocyanins to light, heat, and oxygen; all samples were immediately prepared after extraction for injection into the HPLC as described above.

3. Results & Discussion

To illustrate the advantage of coupling critical fluid-based processes for isolating and purifying natural products, we cite our results for obtaining enriched-phospholipid (PL) extracts and fractions starting from seed oil sources such as soybean. As shown in Figure 3, comminuted soybeans can be initially extracted with either hexane or SC-CO_2 [13], and than countercurrently contacted with SC-CO_2 in a packed fractionationing tower [10] to yield a degummed vegetable oil (VO) while isolating a very enriched lecithin (PL) fraction. The lecithin fraction can than be further fractionated by applying preparative SFC in which a stepwise gradient program facilitates isolation of PLs, such as phosphatidylcholine (PC) or phosphatidylethanol amine at 75% purity levels. Taylor et al. [11] have demonstrated the above process for both enriched lecithin fractions as well as starting from soybeans. It is interesting to note that several SC-CO_2-processed PL extracts are

available today as commercial products.

Hexane or SC-CO_2 Extraction of VO
↓
SC-CO_2 Degumming of VO
↓
Lecithin Isolation
↓
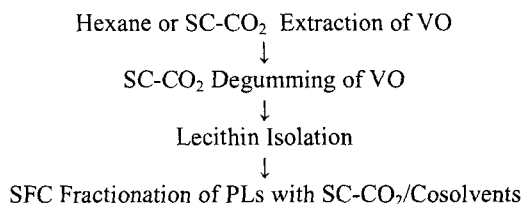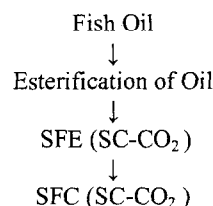SFC Fractionation of PLs with SC-CO_2/Cosolvents

Figure 3. Coupled Processing for Phospholipid Production Using SC-CO_2.

A method has also been developed which permits the production of fish oil esters using the following sequence of steps:

Fish Oil
↓
Esterification of Oil
↓
SFE (SC-CO_2)
↓
SFC (SC-CO_2)

This process has been practiced at a plant located in Tarragona, Spain where fish stocks from European waters have been chemically esterified to their ethyl esters before applying a preliminary SFE step to enrich the polyunsaturated fatty acid (PUFAs) ester fraction. Large scale production SFC is then applied to enrich the various omega fish oil ester fractions using chemically-bonded silica-based packings, yielding a commercial PUFA product of 95% purity. This is an excellent example of a combined SFE-SFC process. It is worth noting that the above chemical esterification could have been conducted in the presence of SC-CO_2 to form the desired ester [14], and thereby creating a SFR-SFE-SFC coupled processing scheme.

Extraction of the berry substrates with sub-H_2O offers another discrete process that can be coupled with SFE using SC-CO_2 or perhaps a SFM option to enrich the aqueous extracts. Extraction of anthocyanins as noted in the Experimental section are frequently done with ethanol or aqueous ethanolic solvents, and must be done with care due to light-, heat-, and air-sensitivity of anthocyanins. Extraction using sub-H_2O is largely dependent on altering the extraction temperature of the fluid above its normal boiling point while under pressure, thereby changing the dielectric constant of water and hence the salvation power of the fluid [15]. For

example, by adjusting temperature and pressure, the dielectric constant of the water at 20°C (~80) can be changed to a value of 48 at 100°C. This is close to the dielectric constant values for furfural (42), glycerol (47) and acetonitrile (38) at 20°C, or methanol (37.5) at 0°C. Hence, sub-H_2O offers an extraction medium that is difficult to match using GRAS (Generally-Regarded-As-Safe) organic solvents and somewhat unique in its extraction characteristics. Evidence of the use of sub-H_2O in the literature for natural products is provided for the extraction of kava-kava [16], rosemary [17], and savory or peppermint [18]. The results presented below in Tables II and III for the acidified sub-H_2O extraction of anthocyanins from berry pomaces, stems, and seeds at 120°C further demonstrate the usefulness of sub-H_2O as an extraction solvent.

The results in Table II indicate that the volume of sub-H_2O required to carry out an equivalent extraction of dried elderberry seeds is much less than when using ethanol as the extraction solvent. Extraction of 90% of the available ANCs from the same substrate (the 90%+Sub-H_2O results) yields a much more concentrated aqueous extract, but takes only 15 min versus the 40 min extraction times associated with the other results. Not only does this reduce the extraction time, but more than half the volume of the required solvent.

Table II. Sub-H_2O Extraction of Elderberry Seeds (Dry).

Solvent	mg ANC/g-seed	g ANC/g-solvent	Ratio
Ethanol	4.76	142	33:1
Sub-H_2O	4.34	213	21:1
90%+Sub-H_2O	4.17	1853	7:1

ANC = Anthocyanin
Ratio = solvent (fluid)/substrate

The above trends are also substantiated by the results shown in Table III for the extraction of black raspberry pomace by sub-H_2O. The pomace is the substance left over after the removal of the juice from black raspberries. Here, extraction with ethanol yields an approximately equivalent result to that obtained on dried elderberry seeds (Table II). The results for extraction with sub-H_2O and 90%+ Sub-H_2O yield less total ANC than in the case of the whole dried elderberry seeds, but this is due to the reduced levels of ANCs found in all pomaces after the juice is expressed.

Table III. Sub-H$_2$O Extraction of Black Raspberry Pomace.

Solvent	mg ANC/g-seed	g ANC/g-solvent	Ratio
Ethanol	4.79	141	35:1
Sub-H$_2$O	3.85	137	28:1
90%+Sub-H$_2$O	3.50	237	15:1

ANC = Anthocyanin
Ratio = solvent (fluid)/substrate

Similar encouraging results have been achieved with moist elderberry seeds, elderberry stems, and blueberry pomaces. It should be noted that the dried and moist elderberry substrates contained between 7.4 – 9.3 % moisture, while the raspberry and blueberry pomaces contained approximately 65% moisture. The above recoveries of anthocyanins at an extraction temperature of 120°C might seem somewhat surprising considering their inherent thermal instability, however calculations of the superficial velocity of sub-H$_2$O through the extraction cell are very rapid (~0.1 cm/sec), facilitating rapid mass transport of the target solutes (ANCs) from the substrate. One additional benefit of the "hot" water extraction process is in-situ sterilization of resultant product, thereby potentially avoiding the need for thermal retorting of the final product. These advantages have resulted in the filing of a patent application recently by [19] for the above process.

Average percentages of ANCs in the final aqueous extract ranged from 8-10% for the extraction of berry seeds/stems to 2.5-4.5% from the pomaces. Although the tintorial strength of such extracts is high, it would be desirable to further concentrate these extracts for applications in the nutraceutical or functional food areas. This could nicely be accomplished by coupling a SFM process step after sub-H$_2$O to yield a SFE-SFM coupled process. It should be noted that the use of SFE with SC-CO$_2$ (neat and with cosolvents) has been reported in the literature for extracting both the oil and enriched polyphenolic fractions from grapes [20,21,22]. Such results suggest that by combing sequential extractions using SC-CO$_2$ and than sub-H$_2$O, that an array of useful natural product extracts could be obtained, as noted by author previously [23].

4. Conclusions

Total critical fluid coupled processes can be developed by combining discrete SFE, SFF, and SFR steps with SC-CO$_2$ and other pressurized fluids. Using this approach, a variety of useful target products can be developed that are not accessible using one critical fluid-based process alone. An additional advantage of using multiple critical fluid processing steps is that it can help offset the capitalization costs that are required in constructing a high pressure processing plant, i.e., permitting more universal application of the SC-CO$_2$ delivery and recycle system. Some additional benefits can also occur when using high pressure carbon dioxide or water for processing natural products, such as the deactivation of harmful microbes or enzymes, allowing sterilization of the end products as occurs at much higher pressures and temperatures in ultra-high pressure food processing [24].

References

1. P.G. Jessop and W. Leitner (eds.), 'Chemical Synthesis Using Supercritical Fluids', VCH-Wiley, Weinheim, 1995.

2. J.W. King, 'Supercritical fluid technology for lipid extraction, fractionation, and reactions', in T.M. Kuo and H.W. Gardner (eds.). Lipid Biotechnology, Marcel Dekker, NY 2002, p. 663.

3. E. Reverchon, 'Supercritical fluid extraction and fractionation of essential oils and related products', J. Supercritical Fluids, 1997, 10, 1.

4. A. A. Clifford, A. Basile, M.M. Jimenez-Carmona, and S.H.R. Al-Saidi, 'Extraction of natural products with superheated water'. Proceedings of the 6th Meeting on Supercritical Fluids, Nottingham, UK, 1999, p. 485.

5. R.W. Towsley, J. Turpin, M. Sims, J. Robinson, and W. McGovern, 'Porocritical fluid extraction using CO$_2$ for industrial recovery and recycle', Proceedings of the 6th Meeting on Supercritical Fluids, Nottingham, UK, 1999, p. 579.

6. F. Temelli, J.W. King, and G.R. List, 'Conversion of oils to monoglycerides by glycerolysis in supercritical carbon dioxide media', J.Am. Oil Chem. Soc., 1996, 73, 699.

7. M.A. Jackson and J.W. King, 'Lipase-catalyzed glycerolysis of soybean oil in supercritical carbon dioxide', *J. Am. Oil Chem. Soc.*, **1997**, 74, 103.

8. J.W. King, R.L. Holliday, E. Sahle-Demessie, F.J. Eller, and S.L. Taylor, 'Extraction, fractionation, and reaction of agricultural, food, and natural products using supercritical carbon and cosolvent mixtures'. Proceedings of the 4th International Symposium on Supercritical Fluids, **1997**, 10, p. 833.

9. M.B.O. Andersson, J.W. King and L. G. Blomberg, 'Synthesis of fatty alcohol mixtures from oleochemicals in supercritical fluids', Green Chem., **2000**, 2, 230.

10. G.R. List, J.W. King, J.H. Johnson, K. Warner, and T.L. Mounts, 'Supercritical CO_2 degumming and physical refining of soybean oil', *J. Am. Oil Chem. Soc.*, **1993**, 70, 473.

11. S.L. Taylor, J.W. King, Montanari, P. Fantozzi, and M.A. Blanco, 'Enrichment and fractionation of phospholipid concentrates by supercritical fluid extraction and chromatography', *Ital. J. Food Sci.*, **2000**, 12, 65.

12. G. Skrede, R.E. Wrolstad, and R.W. Durst, 'Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (V*accinium melanocarpa*)', *J. Food Sci.*, **2000**, 65, 357.

13. J.P. Friedrich, G.R. List, and A.J. Henken, 'Petroleum-free extraction of oil from soybeans with supercritical CO_2', *J. Am. Oil Chem. Soc.*, **1982**, 59, 288.

14. M.A. Jackson and J.W. King, 'Methanolysis of seed oils in flowing supercritical carbon dioxide', *J. Am Oil Chem. Soc.*, **1996**, 73, 353.

15. G. Akerof, 'Dielectric constants of some organic solvent-water mixtures at various temperatures', *J .Am. Chem. Soc.*, **1932**, 54, 4125.

16. A. Kubatova, D.J. Miller, and S.B. Hawthorne, 'Comparison of subcritical water and organic solvents for extracting kava lactones from kava root', *J .Chromatogr. A*, **2001**, 923, 187.

17. A. Basile, M.M. Jimenez-Carmona, and A.A. Clifford, 'Extraction of rosemary by superheated water', *J. Agric. Food Chem.*, **1998**, 46, 5205.

18. A. Kubatova, A.J.M. Lagadec, D.J. Miller, and S.B. Hawthorne, 'Selective extraction of oxygenates from savory and peppermint using subcritical water', *Flavor Fragrance J.*, **2001**, 16, 64.

19. J.W. King and R. Grabiel, 'Isolation of polyphenolic compounds from fruits or vegetables utilizing subcritical water extraction', U.S. Patent Application #10/171258, **2002**.

20. M. Palma and L.T. Taylor, 'Extraction of polyphenolic compounds from grape seeds with near critical carbon dioxide', *J Chromatogr. A .*, **1999**, 849, 117.

21. R. Murga, M. T. Sanz, S. Beltran, and J.L, Cabezas, 'Solubility of some phenolic compounds contained in grape seeds, in supercritical carbon dioxide', *J. Supercritical Fluids*, **2002**, 23, 113.

22. H. Sovova, J. Kucera, and J. Jez, 'Rate of the vegetable oil extraction with supercritical CO_2 - extraction of grape oil', *Chem. Eng. Sci.,*. **1994**, 49, 415.

23. J.W. King,. 'Advances in critical fluid technology for food processing', *Food Sci. Techn. Today*, **2000**, 14, 186.

24. S. Grant, M. Patterson, and D. Ledward, 'Food processing gets freshly squeezed', *Chem. Ind.*, **2000**, No. 2, 489.